

New developments in FcεRI regulation, function and inhibition

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Abstract | The high-affinity Fc receptor for IgE (FcεRI), a multimeric immune receptor, is a crucial structure for IgE-mediated allergic reactions. In recent years, advances have been made in several important areas of the study of FcεRI. The first area relates to FcεRI-mediated biological responses that are antigen independent. The second area encompasses the biological relevance of the distinct signalling pathways that are activated by FcεRI; and the third area relates to the accumulated evidence for the tight control of FcεRI signalling through a broad array of inhibitory mechanisms, which are being developed into promising therapeutic approaches.

Immunoreceptor tyrosine-based activation motif (ITAM). A structural motif containing tyrosine residues, found in the cytoplasmic tails of several signalling molecules. The motif has the form Tyr-X-X (Leu/Ile), where X denotes any amino acid, and the tyrosine is a target for phosphorylation by SRC tyrosine kinases and subsequent binding of proteins containing SH2 domains.

Allergic rhinitis, asthma, atopic dermatitis, and food and drug allergies, which in some cases can lead to life-threatening anaphylaxis, are an increasing problem. These conditions are often a result of IgE-mediated allergic reactions, the pathophysiology of which involves the high-affinity Fc receptor for IgE (FcεRI). As we discuss in this Review, recent advances have been made in the understanding of antigen-independent regulatory mechanisms in FcεRI biology. FcεRI cell-surface expression, a crucial aspect for the outcome of allergic reactions, is positively regulated by the FcεRI ligand, IgE, and by the β-chain of FcεRI, whereas an alternatively spliced variant of the β-chain can counteract this expression. In addition, IgE binding to FcεRI can lead in some cases to low-level receptor aggregation and antigen-independent effects. In addition, two distinct pathways of FcεRI signalling have been described and further dissected. Finally, new inhibitory receptors for FcεRI signalling and their mechanisms have been described, as well as novel therapeutic approaches that take advantage of the function of inhibitory receptors.

Structure of FcεRI

FcεRI is a multimeric cell-surface receptor that binds the Fc fragment of IgE with high affinity. It exists as a tetrameric and trimeric complex (see below and FIG. 1). In its complete tetrameric form, FcεRI contains an α-chain (FcεRIα), a β-chain (FcεRIβ) and a homodimer of disulphide-linked γ-chains (FcεRIγ; here, the tetramer is referred to as αβγ₂). The structure of FcεRI is described briefly here to enhance our understanding of the recent data on FcεRI regulation.

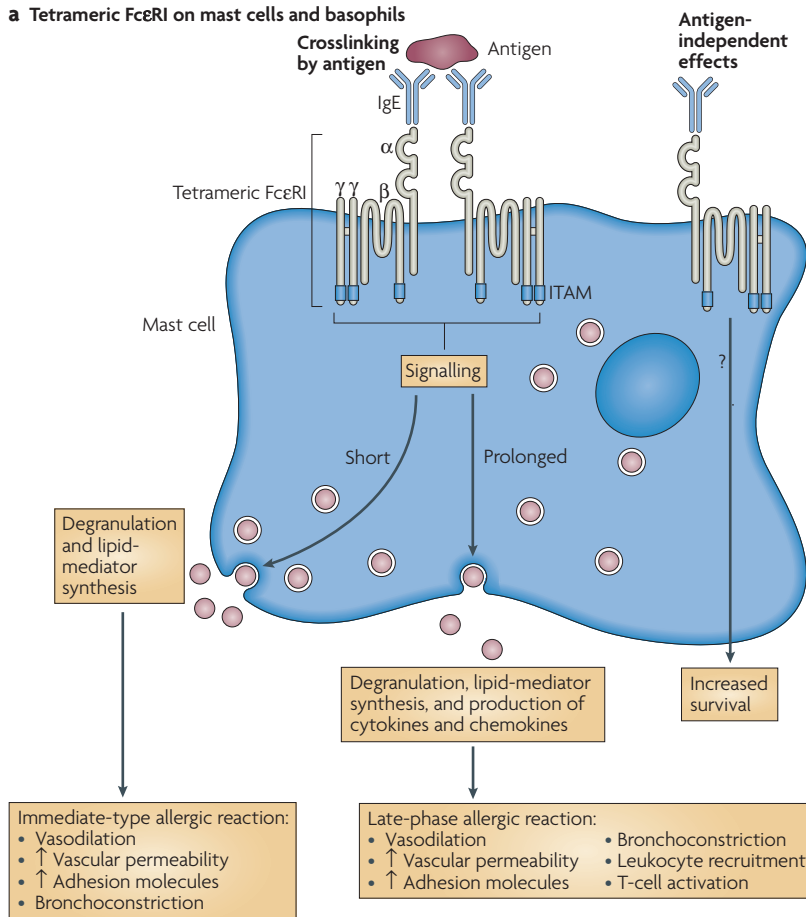
The α-chain belongs to the immunoglobulin superfamily and comprises two extracellular immunoglobulin-related domains that bind a single IgE molecule, a transmembrane domain that contains a conserved aspartic-acid residue and a short cytoplasmic tail¹. The human α-chain is N-glycosylated at seven sites. Glycosylation is required to mediate proper interaction between the α-chain and the folding machinery in the endoplasmic reticulum (ER)². The intracellular tail of the α-chain has no apparent signalling function¹.

The extracellular region of the α-chain has been crystallized as an isolated protein and in a complex with the Fc fragment of an IgE^{3–7}. Unexpectedly, a single FcεRI uses two asymmetrical sites to bind the dimeric and symmetrical Fc fragment, thereby mandating a ligand:receptor stoichiometry of 1:1. This structure of the complex explains the high affinity of FcεRI for IgE as a result mainly of the low dissociation rate. This low dissociation rate is given by the increased capacity of each dissociated receptor site to rebinding IgE when the other site is still occupied by IgE. So, each receptor site has an intrinsic affinity (probably in the micromolar range) that is much lower than the overall receptor affinity. This finding is important for the development of strategies to interfere with the IgE–FcεRI interaction. One can predict that a drug that interferes with one of the binding sites would be sufficient to block binding by inducing IgE dissociation.

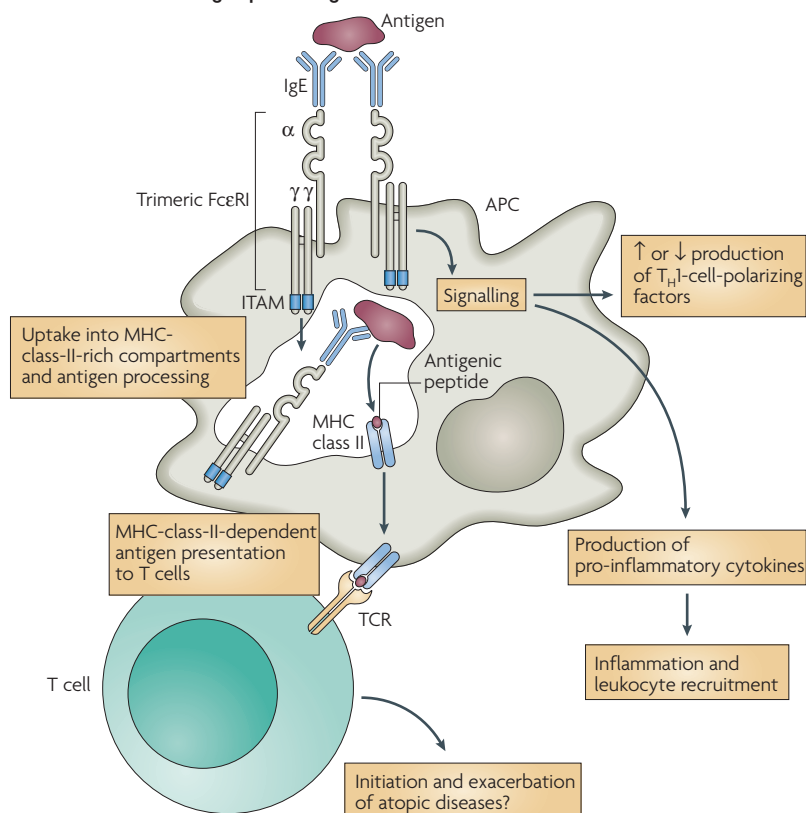
The FcεRI β- and γ-chains have no role in ligand binding¹. These chains each have an immunoreceptor tyrosine-based activation motif (ITAM), which is tyrosine

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a Tetrameric FcεRI on mast cells and basophils



b Trimeric FcεRI on antigen-presenting cells



phosphorylated after antigen crosslinking of receptor-bound IgE molecules. This results in the association of the β- and γ-chains with intracellular signalling molecules through their SRC homology 2 (SH2) domains⁸. Whereas the human β-chain is an amplifier of FcεRI signals and cell-surface expression, the γ-chains are the main and indispensable FcεRI signalling units¹. The γ- and β-chains are shared with other Fc receptors. For example, the high- and low-affinity Fc receptors for IgG (FcγRI and FcγRIII, respectively), and the Fc receptor for IgA (FcαRI) associate with the γ-chain depending on the cellular context¹.

Regulation of FcεRI expression

Whereas murine FcεRI is confined to the surface of mast cells and basophils, there is a wider distribution of FcεRI in humans. Monocytes, Langerhans cells, eosinophils and dendritic cells (DCs) express trimeric αγ₂ FcεRI complexes¹ (the differences between human and murine FcεRI are summarized in TABLE 1). The expression of FcεRI, with unclear subunit composition, has been reported on human platelets and neutrophils^{9–11}.

Figure 1 | Composition of the FcεRI complex (tetrameric and trimeric structures) and biological functions. a | The tetrameric form of the high-affinity Fc receptor for IgE (FcεRI) consists of one IgE-binding α-chain with two immunoglobulin-like domains (depicted by the loops in the extracellular part), one β-chain and two γ-chains with intracellular immunoreceptor tyrosine-based activation motifs (ITAMs) for signal transduction. Tetrameric FcεRI is expressed by mast cells and basophils. Upon crosslinking of FcεRI by IgE and antigen, a signalling cascade leads within minutes to the release of preformed mediators, such as histamine, and lipid-mediator synthesis. These events produce an immediate-type allergic reaction characterized by vasodilation, increased vascular permeability, upregulation of vascular adhesion molecules and bronchoconstriction. Upon prolonged stimulation, a late-phase allergic reaction is induced, which in addition leads to cytokine and chemokine production, inducing the recruitment of inflammatory cells and T-cell activation. With continuous or repetitive stimulation in chronic inflammation, FcεRI-bearing mast cells and basophils can contribute to the features of the specific inflammatory response (such as bronchial hyper-reactivity). Antigen-independent effects are mediated by IgE binding to FcεRI, and may result in increased survival of the cell. **b |** Trimeric FcεRI lacks the β-chain and is expressed by antigen-presenting cells (APCs; and eosinophils). Crosslinking of FcεRI by IgE and antigen leads to the endocytosis of FcεRI and IgE-bound antigen, followed by their uptake in MHC-class-II-rich compartments, in which antigen processing and loading of antigenic peptides onto MHC class II molecules occurs, and ultimately to the presentation of the antigenic-peptide–MHC-class-II complexes to T cells. This is an efficient process for allergen presentation, although its causal role in atopic diseases remains to be determined. FcεRI crosslinking also induces signalling, which leads to the production of pro-inflammatory cytokines and, depending on the APC subtype, the upregulation or downregulation of production of T helper 1 (T_H1)-cell-polarizing factors. TCR, T-cell receptor.

SRC homology 2 (SH2) domain

A protein domain that is commonly found in signal-transduction molecules. It interacts specifically with phosphotyrosine-containing peptides.

Table 1 | Comparison of human and murine FcεRI

Property	Murine FcεRI*	Human FcεRI*
Structure	Tetrameric αβγ ₂ structure only	Tetrameric αβγ ₂ structure and trimeric αγ ₂ structure
Expression pattern	Mast cells and basophils	Mast cells, basophils, monocytes and macrophages, myeloid dendritic cells, plasmacytoid dendritic cells, Langerhans cells, eosinophils and platelets
Regulation of expression	IL-4 does not enhance α-chain expression	IL-4 enhances α-chain expression
Binding properties	Binds murine IgE	Binds human and murine IgE

*REFS 1, 9–11, 13–17. FcεRI, high-affinity Fc receptor for IgE; IL-4, interleukin-4.

As the binding of IgE to cell-surface FcεRI is the first step in a cascade of events leading to allergic reactions, the amount of FcεRI expressed on the surface of cells determines how much allergen-specific IgE, and thereby allergens, gain access to effector cells to initiate an allergic reaction. Cell-surface expression of the FcεRI complex is regulated through the availability of specific receptor components and by the amount of its ligand, IgE (FIG. 2).

Regulation of α-chain expression and co-expression of β- and γ-chains. Assembly, maturation and transport of the FcεRI complex to the cell surface occur as follows¹. Non-covalent association of the newly synthesized FcεRIα core protein with FcεRI β- and γ-chains occurs co-translationally in the ER¹². This association supports core glycosylation of FcεRIα, which is required for proper folding, signal-peptide cleavage, export from the ER¹² and subsequent cell-surface expression². The FcεRI

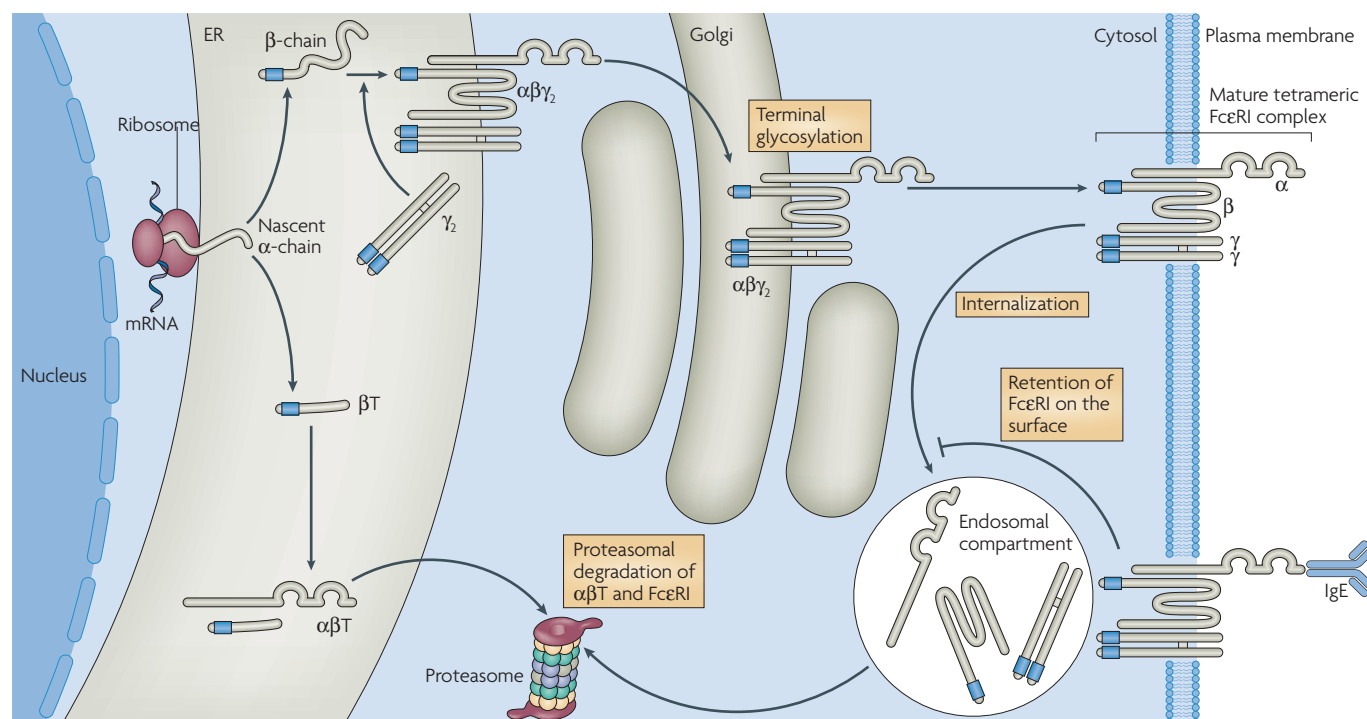


Figure 2 | Regulation of human FcεRI cell-surface expression by mast cells and basophils. In humans, interleukin-4 (IL-4) induces the production of immature core-glycosylated α-chains of the high-affinity Fc receptor for IgE (FcεRI) in the endoplasmic reticulum (ER). In the absence of other FcεRI chains, the immature α-chain remains in the ER. Unknown signals lead to the co-translational production of γ-chains and of the classical full-length β-chain. The γ-chains mask an ER retention signal in the α-chain, leading to the export of the tetrameric structure (αβγ₂) or of the trimeric structure of the FcεRI (αγ₂; not shown) from the ER. This is followed by full maturation of the FcεRI by terminal glycosylation in the Golgi compartment, and finally its transport to the cell surface; this process is more efficient for the tetrameric complexes, which are also more stable on the cell surface than those complexes that lack the β-chain. The FcεRI ligand, IgE, stabilizes FcεRI complexes on the cell surface and limits their internalization. In the presence of ongoing FcεRI synthesis, this inhibition of FcεRI internalization leads to the accumulation of more FcεRI complexes on the cell surface. However, if the truncated β-chain variant, βT, is produced by alternative splicing, this prevents the FcεRI complex from reaching the cell surface and leads to proteasomal degradation of immature FcεRI complexes.

complex containing the immature α -chain is then transferred to the Golgi compartment for terminal glycosylation. The mature Fc ϵ RI complex is then transported to the cell surface.

In human mast cells and antigen-presenting cells (APCs), the T helper 2 (T_H2)-cell-derived cytokine interleukin-4 (IL-4) can induce Fc ϵ RI α expression^{13–16}. A new study of the distal human *FCER1A* promoter confirmed that IL-4 increases the intracellular expression of Fc ϵ RI α (REF. 17). A comparable IL-4-sensitive promoter region is absent in the murine *FCER1A* gene, which explains why IL-4 cannot enhance Fc ϵ RI α expression in murine cells. Thus, the presence of the T_H2-type cytokine IL-4 is a prerequisite for Fc ϵ RI expression by human cells.

The atopic status of humans correlates with the surface levels of Fc ϵ RI on mast cells, basophils, monocytes and DCs^{18–22}. Co-expression with the Fc ϵ RI γ -chains is a requirement for the Fc ϵ RI α -chain to reach the surface and ultimately for the Fc ϵ RI complex to function (FIG. 2). In the absence of γ -chains, the α -chain is retained in its immature form in the ER^{14,23–25}. Early transfection studies have indicated that the γ -chain masks an ER-retention motif in the α -chain, and this leads to export and maturation of the Fc ϵ RI complex²⁶.

Rodents and humans differ in their β -chain requirement for Fc ϵ RI cell-surface expression (TABLE 1). Murine Fc ϵ RI has an obligatory tetrameric $\alpha\beta\gamma_2$ structure²⁷. Co-expression of the β -chain is essential for cell-surface expression of Fc ϵ RI, which suggests additional ER-retention signal(s) in murine Fc ϵ RI α that are masked on association with the Fc ϵ RI β -chain. The ER-retention signal might be located in the extracellular domain of the murine α -chain²⁸.

By contrast, co-expression of the Fc ϵ RI β -chain is dispensable for Fc ϵ RI cell-surface expression in humans. In humans, both $\alpha\gamma_2$ and $\alpha\beta\gamma_2$ complexes are present on the cell surface of mast cells and basophils, and $\alpha\gamma_2$ complexes are present on APCs and eosinophils¹. As the tissue distribution of the β -chain is highly restricted, this might explain the broader tissue distribution of human Fc ϵ RI. Classical full-length Fc ϵ RI β acts as an amplifier of Fc ϵ RI cell-surface expression on human cells by promoting maturation and trafficking of associated Fc ϵ RI α and possibly by stabilizing the cell-surface-expressed Fc ϵ RI complex²⁹. To demonstrate the amplifier role of Fc ϵ RI β in this process, we have shown a correlation between the presence of the Fc ϵ RI β protein and the cell-surface expression of the whole complex²⁹. Introduction of Fc ϵ RI β into $\alpha\gamma_2$ -expressing cells resulted in a significant increase in the expression levels of Fc ϵ RI on the cell surface; this might account for the large difference in surface density of Fc ϵ RI between Fc ϵ RI β -negative cells (monocytes and DCs) and Fc ϵ RI β -positive cells (mast cells and basophils) *in vivo*.

Role of the truncated β -chain variant β T. Interestingly, the gene encoding Fc ϵ RI β also produces an alternatively spliced product that has an antagonistic function. This transcript retains the fifth intron, which contributes an extra 16-amino-acid sequence that replaces the normal

fourth transmembrane domain and carboxyl terminus of the β -chain. The splice variant produces a truncated protein termed β T³⁰. In transfectants, β T protein has an extremely short half-life due to its rapid proteasomal degradation. β T inhibits Fc ϵ RI cell-surface expression, as $\alpha\beta$ T γ_2 transfectants express less cell-surface Fc ϵ RI than do $\alpha\gamma_2$ transfectants. β T mediates this effect by inhibiting α -chain maturation. It associates with nascent α -chain and sequesters it away from its normal maturation pathway (FIG. 2). This is accompanied by the accumulation of $\alpha\beta$ T γ_2 complexes in the ER, which contain non-glycosylated 'backbone' Fc ϵ RI α with an uncleaved signal peptide¹². When β T is expressed by transfection of cells expressing $\alpha\beta\gamma_2$, it competes with the classical β -chain for binding to the α -chain and decreases Fc ϵ RI cell-surface expression. In human basophils, β T and the classical β -chain are co-expressed in variable proportions³⁰, which indicates that the β T: β -chain ratio could regulate Fc ϵ RI cell-surface expression and thereby influence susceptibility to allergic disorders.

Role of IgE. The first observation of the potential regulation of Fc ϵ RI by its ligand IgE was made when Malveaux *et al.*¹⁸ found a correlation between serum IgE levels and the amount of IgE bound to basophils. Later, monomeric IgE was shown to increase Fc ϵ RI expression on the cell surface^{31,32}. The low level of expression of Fc ϵ RI on mast cells from IgE-deficient mice could be markedly upregulated by incubation with IgE *in vitro* or by injection of IgE *in vivo*³³. Other studies confirmed similar effects of IgE on the levels of surface Fc ϵ RI on basophils, mast cells, monocytes and DCs^{14,34–36}. Conversely, the treatment of atopic patients with IgE-specific antibodies leads to decreased levels of serum IgE and of Fc ϵ RI expression on basophils³⁴. Importantly, this IgE-mediated upregulation of Fc ϵ RI expression leads to increased effector-cell functions, such as mast-cell mediator release and IgE-dependent antigen-presenting functions of DCs^{14,33,35,36}.

Mechanistically, IgE-mediated upregulation of mast-cell Fc ϵ RI expression results from stabilization of receptors on the cell surface by the ligand. This stabilization stops degradation of cell-surface-expressed receptors while maintaining basal synthesis. This, in turn, leads to the progressive accumulation of receptors at the cell surface (FIG. 2). Initially, the accumulation comes from the use of a preformed receptor pool derived from recycled and recently synthesized receptors³⁷. This early phase is insensitive to the protein-synthesis inhibitor cycloheximide³³. Later on, further receptor accumulation at the cell surface depends on a basal level of Fc ϵ RI protein synthesis and becomes sensitive to cycloheximide^{33,37}. This stabilization mechanism applies to both $\alpha\gamma_2$ and $\alpha\beta\gamma_2$ complexes³⁷. Note that any treatment that affects the basal synthesis of Fc ϵ RI (such as IL-4) would indirectly have an effect on cell-surface receptor upregulation. However, there is no evidence for an IgE-mediated increase in transcription or translation of Fc ϵ RI. Monomeric IgE can also induce the upregulation of cell-surface expression of trimeric Fc ϵ RI by DCs by a comparable mechanism¹⁴. The structural basis for the receptor stabilization by IgE remains to be defined.

Biological functions of FcεRI

FcεRI expressed on the surface of mast cells and basophils is well known as a key regulator of IgE-mediated immediate-type allergic reactions and late-phase allergic reactions (FIG. 1). This is reflected by the release of preformed mediators such as histamine, heparin, neutral proteases and others, the generation of leukotrienes and prostaglandins, and the *de novo* synthesis of cytokines and chemokines upon crosslinking of FcεRI-bound IgE by antigen^{38,39}. *In vivo*, IgE-mediated local or systemic anaphylaxis is abolished in mice deficient in FcεRI α- or β-chains. Components of IgE-dependent late-phase responses, such as tissue swelling, fibrin deposition and leukocyte accumulation, also depend on FcεRI-bearing mast cells and basophils^{40,41}. This topic and the role of FcεRI-bearing cells in host defence and asthma have been reviewed extensively elsewhere^{1,38,42}. Recently, antigen-independent effects of IgE bound to FcεRI have been of considerable interest, and the role of FcεRI in contact hypersensitivity, as well as on different types of APC has been defined more clearly.

Antigen-independent effects. Two studies^{43,44} have shown that IgE alone — without antigen — can enhance mast-cell survival upon growth-factor deprivation. This provides an attractive explanation for how increased serum IgE levels (as observed in allergic patients) could enhance allergic reactions — by increasing the amount and viability of allergic effector cells (and by increasing FcεRI levels). Interestingly, a later study confirmed that transplantation of an IgE-producing hybridoma into mice increased the number of gastric mast cells⁴⁵.

Whereas Kalesnikoff *et al.*⁴⁴ observed IgE-induced FcεRI signalling (activation of extracellular-signal regulated kinase (ERK), JUN N-terminal kinase (JNK), p38 and AKT (also known as PKB) signalling), and postulated that autocrine secretion of cytokines and induction of the anti-apoptotic protein B-cell lymphoma X_L (BCL-X_L) are responsible for the enhanced mast-cell survival, Asai *et al.*⁴³ did not observe any of these signalling events. Other studies have since shown that different IgE clones were responsible for these divergent results and that so-called 'highly cytokinergic' IgE clones (such as those used by Kalesnikoff *et al.*) can produce some FcεRI aggregation without crosslinking by antigen. Incubation with these IgE clones produces a broad range of FcεRI-induced events, such as histamine synthesis and release, adhesion, migration and DNA synthesis, through the induction of signalling events that are known to occur after 'classical' FcεRI crosslinking by IgE and antigen (such as activation of spleen tyrosine kinase (SYK))^{45–48}. Autocrine IL-3 production, together with BCL-X_L and BCL-2 upregulation, seems to be a major component of enhanced mast-cell survival by highly cytokinergic IgE⁴⁹.

Importantly, the effects of 'monomeric' IgE, other than FcεRI upregulation, can be inhibited by hapten administration, which shows that these effects are due to low-level FcεRI aggregation^{47,50,51}. Whether low-level FcεRI aggregation due to monomeric IgE, for example in the case of very high serum IgE concentrations, is

physiologically or pathologically relevant is a question that remains open.

FcεRI on antigen-presenting cells. In humans, trimeric FcεRI αγ₂ is expressed by professional APCs such as DCs (including epidermal Langerhans cells), monocytes and macrophages (for more detail see REFS 1,52). FcεRI surface expression by these cells is upregulated in atopic donors and — for DCs and Langerhans cells — correlates with serum IgE levels as a marker of disease activity. Indeed, data indicate that FcεRI expressed by APCs has a role in the pathogenesis and effector phase of allergic disease. Maurer *et al.*^{19,53,54} showed that monocytes and DCs from allergic patients present birch-pollen allergen to T cells more efficiently when specific IgE is added, which leads to the channelling of IgE–FcεRI-bound antigen into intracellular MHC-class-II-rich compartments and the presentation of antigen-derived peptides. However, proof of a fundamental role of trimeric FcεRI on APCs in the initiation and exacerbation of atopic diseases is still lacking.

There is evidence that FcεRI on APCs, comparable with FcεRI on mast cells and basophils, can have a pro-inflammatory function in allergic patients. FcεRI crosslinking on monocytes, Langerhans cells and DCs from atopic patients can induce protein-tyrosine kinase activation, calcium mobilization and the activation of pro-inflammatory transcription factors such as nuclear factor-κB (NF-κB)^{19,55–57}. This leads to the production of several pro-inflammatory and chemotactic factors, such as tumour-necrosis factor (TNF), CC-chemokine ligand 2 (CCL2; also known as MCP1), IL-1β, IL-8 and IL-16, which can support the inflammatory microclimate in tissues of atopic patients^{56,58–60}. For example, IL-16 production induced by FcεRI crosslinking on Langerhans cells from patients with atopic dermatitis could lead to the recruitment of CD4⁺ T cells, DCs and eosinophils to skin sites of allergen exposure⁵⁸.

Moreover, FcεRI crosslinking on different DC subsets might have different effects on the T_H-cell phenotype. Langerhans cells can help to produce a T_H2-cell phenotype, which is seen in the initial phases of atopic dermatitis, whereas another DC subtype that is present only in established inflammatory skin lesions produces IL-12 and IL-18 upon FcεRI crosslinking and might support the development of the T_H1-cell phenotype, which is usually seen in chronic atopic dermatitis lesions^{61,62}. In addition, it was found recently that FcεRI ligation on plasmacytoid DCs decreases their capacity to secrete interferon-α (IFNα) and IFNβ upon Toll-like receptor 9 (TLR9) stimulation, thereby decreasing their T_H1-cell-polarizing capacity^{61,63}.

FcεRI in delayed-type hypersensitivity reactions. The role of mast cells in delayed-type hypersensitivity (DTH) reactions, such as cutaneous hypersensitivity (CHS) to contact allergens, has been controversial (see REF. 42). However, a role for mast cells and FcεRI in certain aspects of CHS has recently become evident. First, using mast-cell-deficient mice, Biedermann *et al.*⁶⁴ showed that mast-cell-derived TNF and CXC-chemokine

Immediate-type allergic reaction

The IgE-mediated 'type I' immune reaction that occurs within minutes to a few hours of allergen exposure, which can be localized (for example, acute rhinoconjunctivitis, acute asthma attack, urticaria and food allergy) or systemic (anaphylaxis). In this reaction, preformed IgE antibodies bound to FcεRI on mast cells and basophils are crosslinked by allergen, which leads to the release of preformed mediators (for example, histamine), proteolytic enzymes and proteoglycans, as well as lipid mediators and cytokines. These events cause vasodilatation, oedema, fever and bronchoconstriction, leading to the aforementioned clinical conditions.

Late-phase allergic reactions

These reactions are the long-term consequence of mast-cell and/or basophil activation by IgE and allergen. Clinical manifestations can be measurable (visible) two or more hours after allergen exposure but might appear much later. These manifestations peak at 6–9 hours after allergen exposure and have resolved by 24–48 hours. The reactions are characterized by oedema and the infiltration of T helper 2 cells and eosinophils. Tissue reactions are characterized by oedema, pain, warmth and erythema (redness). Reactions in the lungs are characterized by airway narrowing and mucus hypersecretion.

Anaphylaxis

The generalized release of histamine and other inflammatory mediators following systemic induction of mast-cell degranulation by an allergen. Anaphylaxis can cause bronchospasm, cardiovascular collapse and death.

ligand 2 (CXCL2; also known as MIP2; the functional analogue of human IL-8) are essential for the recruitment of polymorphonuclear leukocytes into CHS lesions. However, IgE and FcεRI were not investigated in this study. More recently, Bryce *et al.*⁶⁵ showed that IgE-, mast-cell- and FcγRII-deficient mice (which lack FcεRI and FcγRIII), but not FcγRIII-deficient mice, had markedly decreased CHS reactions and a reduced cellular infiltrate (in particular neutrophils). As CHS reactions could be reconstituted by the administration of IgE molecules of different specificities, this indicates that IgE, through FcεRI on mast cells, has an antigen-independent role in CHS, possibly due to the effects of monomeric IgE on FcεRI expression or cytokine production by mast cells. It is important to note that these mice express FcεRIα on mast cells and basophils only, and not on APCs. The defect in IgE-deficient mice seems to occur in the sensitization phase and is characterized by decreased levels of the mast-cell-derived cytokines mast-cell protease 6 (MCP6), IL-1β, TNF and CCL2, which could explain why the migration of epidermal Langerhans cells upon sensitization in IgE-deficient mice is much less pronounced⁶⁵. An alternative mechanism for IgE–FcεRI-stimulated mast cells to support inflammation in DTH reactions could be through mast-cell-derived leukotrienes. Leukotriene B₄ (LTB₄) that is produced after antigen-dependent FcεRI crosslinking attracts CD8⁺ effector T cells⁶⁶, although it is not clear whether LTB₄ is produced by low-level FcεRI aggregation through monomeric IgE. The induction of expression of vascular cell-adhesion molecule 1 (VCAM1) on endothelial cells or selectins and intercellular adhesion molecule 1 (ICAM1) on leukocytes, for example through mast-cell-derived TNF, is another possibility^{67,68}.

Comparable antigen-independent effects could also contribute to the protection of FcεRIα-deficient mice from trinitrobenzene sulphonic acid (TNBS)-induced colitis, a model of inflammatory bowel disease⁶⁹. Although this is mainly a T_H1-cell-mediated disease, acute early lesions are associated with IL-4 and local IgE production. Transgenic mice overexpressing human FcεRI have higher IL-4 levels and more severe colitis than normal mice. However, the relative contribution of FcεRI on mast cells, basophils and APCs is unclear, as the mice used in this study had an FcεRI tissue distribution equivalent to that of humans (that is, FcεRI expression by mast cells, basophils and APCs).

New developments in FcεRI signal transduction

After antigen crosslinking of FcεRI-bound IgE, a complex intracellular signalling cascade is initiated, which ultimately leads to effector functions (see earlier reviews REFS 1,70–72) (FIG. 3). Recent work has focused on the separation of proximal FcεRI signalling into a primary pathway and a complementary pathway that mainly regulates FcεRI-induced degranulation. The long enigmatic role of sphingosine kinases in FcεRI-induced Ca²⁺ mobilization has been elucidated, and, finally, new adaptors and negative regulators have been described. After a brief review of general principles, these new developments are summarized.

The basic proximal FcεRI signalling events occur as follows. The β- and γ-chains of FcεRI contain ITAMs, which after tyrosine phosphorylation bind the SH2 domains of protein tyrosine kinases (PTKs), mainly the SRC family kinases LYN and FYN, as well as SYK^{1,70,73} (FIG. 3). LYN and FYN are associated with FcεRIβ, and SYK is associated with FcεRIγ. After FcεRI crosslinking, the primary pathway originates from LYN, which is activated and phosphorylates the β- and γ-chain ITAMs. SYK is then recruited to the γ-chain. LYN also transphosphorylates ITAMs in neighbouring FcεRI receptor complexes^{1,70}, resulting in signal amplification. SYK binding to FcεRIγ then leads to LYN-dependent tyrosine phosphorylation and activation of SYK⁷⁴. Among the many substrates for SYK are the adaptor proteins linker for activation of T cells (LAT) and SH2-domain-containing leukocyte protein of 76 kDa (SLP76)^{75,76}. These adaptors then participate in the assembly of large signalling complexes that include other molecules such as the PTK Bruton's tyrosine kinase (BTK), which activates phospholipase Cγ (PLCγ) cooperatively with SYK^{77–79}. In turn, this leads to diacylglycerol (DAG) production and inositol-1,4,5-trisphosphate (InsP₃)-induced Ca²⁺ mobilization⁷⁰.

Primary LYN pathway and complementary FYN pathway. However, studies from LYN-deficient bone-marrow-derived mast cells (BMMCs), which showed normal degranulation, indicated that there is compensation by other molecules in LYN-deficient cells or that a second pathway is involved in degranulation⁸⁰. Newer studies have identified a complementary pathway initiated by FcεRI through FYN and the adaptor growth-factor-receptor-bound protein 2 (GRB2)-associated binding protein 2 (GAB2)^{73,81}. After FcεRI crosslinking, FYN phosphorylates GAB2, which leads to activation of a class I phosphoinositide 3-kinase (PI3K; for more information on PI3K, see BOX 1). This results in activation of the PI3K-dependent kinase 3-phosphoinositide-dependent protein kinase 1 (PDK1) and subsequently of protein kinase Cδ (PKCδ). The LYN pathway is crucial for Ca²⁺ signalling, whereas the FYN pathway is required for degranulation and maintenance and/or amplification of the Ca²⁺ signal. In FYN-deficient cells, PI3K activity and degranulation are impaired, whereas Ca²⁺ signalling is only moderately affected⁷³. By contrast, Ca²⁺ signalling is inhibited in LYN-deficient cells, whereas degranulation is increased, which indicates a negative effect of the LYN pathway on the FYN pathway^{73,80}. There are further indications of crosstalk between the two pathways. Both pathways require SYK activation, as both Ca²⁺ mobilization and degranulation are abolished in SYK-deficient mast cells^{82,83}.

The β-chain of the receptor is known to have a positive effect on FcεRI signalling. Tetrameric FcεRI induced markedly increased FcεRIγ and SYK tyrosine phosphorylation compared with trimeric FcεRI, which lacks the β-chain, as well as a more rapid and increased Ca²⁺ mobilization⁸⁴. The ITAMs in FcεRIβ and FcεRIγ have different structures. The γ-chain ITAM has the

Plasmacytoid DCs

A subset of DCs that was named 'plasmacytoid' because their appearance under the microscope is similar to that of plasmablasts. In humans, these DCs can be derived from lineage-negative haematopoietic stem cells from the peripheral blood. These DCs are the main producers of type I interferons in response to viral infections. They express high levels of CD123 (IL-3 receptor α-chain), high levels of MHC class II, and lack expression of CD11c.

Delayed-type hypersensitivity (DTH)

A cellular immune response to antigen that develops over ~24–72 hours with the infiltration of T cells and monocytes, and is dependent on the production of T-helper-1-specific cytokines.

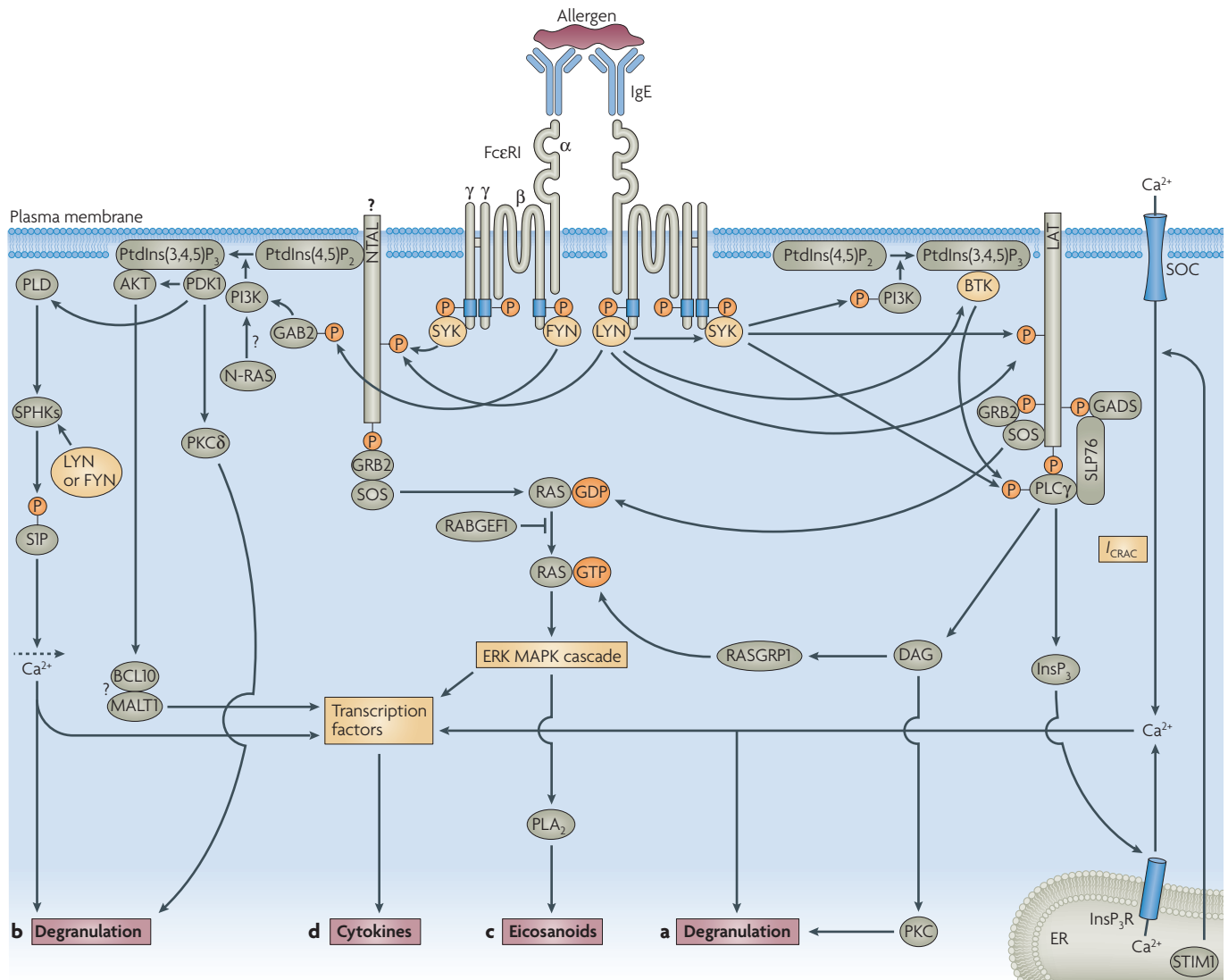


Figure 3 | Simplified model of FcεRI signalling. **a** | In the primary pathway, LYN is activated and phosphorylates immunoreceptor tyrosine-based activation motifs (ITAMs) in the β- and γ-chains of the high-affinity Fc receptor for IgE (FcεRI), which leads to SYK association with γ-chains and subsequent SYK activation. SYK phosphorylates LAT (linker for activation of T cells), which recruits GADS (growth-factor-receptor-bound protein 2) (GRB2)-related adaptor protein, SLP76 (SH2-domain-containing leukocyte protein of 76 kDa), VAV (not shown) and PLCγ (phospholipase Cγ). PLCγ activation occurs through this complex and through PI3K (phosphoinositide 3-kinase)-mediated membrane recruitment of BTK (Bruton's tyrosine kinase) and phosphorylation of PLCγ by BTK. Activated PLCγ produces DAG (diacylglycerol), which activates classical PKCs (protein kinases C), and InsP₃ (inositol-1,4,5-trisphosphate), which induces Ca²⁺ release from the ER (endoplasmic reticulum) through InsP₃ receptors (InsP₃Rs). Store depletion then leads to STIM1 (stromal interaction molecule 1)-guided opening of the store-operated channel (SOC), which produces an extracellular Ca²⁺ influx and I_{CRAC} (calcium-release-activated current). Classical PKC activation and Ca²⁺ mobilization ultimately lead to degranulation. **b** | In the complementary pathway, FYN and SYK activation induce a signalling complex with VAV (not shown), GRB2, GAB2 (GRB2-associated binding protein 2) and PI3K, possibly organized by NTAL (non-T-cell activation linker). PI3K activation leads to the membrane recruitment of PDK1 (3-phosphoinositide-dependent protein kinase 1), which then activates PKCδ, ultimately leading to degranulation. Sphingosine kinases (SPHKs) can be activated by PLD (phospholipase D), LYN or FYN. SPHK activation leads to extracellular Ca²⁺ influx, probably by relieving the suppressive effect of sphingosine on the I_{CRAC}. **c** | GRB2 and SOS (son-of-sevenless homologue) are associated with both the primary and complementary pathways and lead to RAS activation, which then leads to the activation of the ERK (extracellular-signal-related kinase) MAPK (mitogen-activated protein kinase) cascade and eicosanoid production via PLA₂. **d** | Cytokine production is regulated by various transcription factors, the activation of which is dependent on Ca²⁺, PKC and AKT signals (via BCL-10/MALT1?). BCL-10, B-cell lymphoma-10; MALT1, mucosa-associated-lymphoid-tissue lymphoma-translocation protein 1; PtdIns(3,4,5)P₃, phosphatidylinositol-3,4,5-trisphosphate; PtdIns(4,5)P₂, phosphatidylinositol-4,5-bisphosphate; RABGEF1, RAB guanine-nucleotide-exchange factor 1; RASGRP1, RAS guanyl-nucleotide-releasing protein 1; S1P, sphingosine 1-phosphate.

Box 1 | **PI3K is an essential component of FcεRI signalling**

Phosphoinositide 3-kinase (PI3K) seems to have a central, if very complex and not completely understood, role in high-affinity Fc receptor for IgE (FcεRI) signalling. Inhibitory signals often affect PI3K or its products (for a more detailed review of its role in FcεRI signalling see REFS 71, 72). PI3K seems to have an important role in the complementary FYN–GAB2 (growth-factor-receptor-bound protein 2 (GRB2)-associated binding protein 2) pathway through its product phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P₃), which is present in the plasma membrane. PtdIns(3,4,5)P₃ recruits pleckstrin-homology-domain-containing molecules to the plasma membrane so that they can interact with other signalling molecules. For example, it recruits 3-phosphoinositide-dependent protein kinase 1 (PDK1) leading to protein kinase Cδ (PKCδ) activation that regulates mast-cell degranulation. It also leads to AKT activation (through the recruitment of AKT, as well as AKT activation by PDK1), leading to anti-apoptotic effects and the induction of cytokine production. In addition, PI3K seems to have a role in the maintenance of the Ca²⁺ signal through the membrane recruitment of Bruton's tyrosine kinase (BTK), which activates phospholipase Cγ (PLCγ) in cooperation with spleen tyrosine kinase (SYK), thus leading to inositol-1,4,5-trisphosphate (InsP₃) generation⁷². Different PI3K isoforms might be responsible for these different roles¹⁴⁷. The p110γ catalytic subunit of PI3K is linked to G-protein-coupled receptors, such as the adenosine receptor, and can enhance FcεRI-induced Ca²⁺ mobilization and degranulation¹⁴⁸. However, the crucial PI3K catalytic subunit for FcεRI signalling and immediate-type allergy seems to be p110δ. Mice with a point mutation in p110δ have a strong deficit in FcεRI-induced mast-cell degranulation and activation of the FYN-pathway component AKT¹⁴⁹. PI3K is subject to tight cellular control, which is mainly achieved by enzymes that degrade its product PtdIns(3,4,5)P₃. The inositol phosphatase SRC-homology-2-domain-containing inositol-5-phosphatase (SHIP) removes the 5' phosphate from PtdIns(3,4,5)P₃, thereby producing phosphatidylinositol-3,4-bisphosphate (PtdIns(3,4)P₂) (REFS 78, 79). Phosphatase and tensin homologue (PTEN) degrades PtdIns(3,4,5)P₃ to PtdIns(4,5)P₂, the substrate for PLCγ. PTEN-deficient mast cells have increased levels of PtdIns(3,4,5)P₃ and constitutively secrete cytokines¹⁵⁰.

consensus ITAM sequence, (Asp/Glu)-X-X-Tyr-X-X-Leu-(X)_{7–11}-Tyr-X-X-(Leu/Ile), where X denotes any amino acid, whereas the ITAM of the β-chain contains a third tyrosine in its spacer region and the spacing between the canonical tyrosine residues is shortened. More recent studies show that this leads to different interactions with signalling molecules. In fact, the amino-terminal canonical tyrosine (Tyr219) in the FcεRIβ ITAM is essential for the amplifying effect of FcεRIβ, possibly by association with LYN upon FcεRI triggering. Loss of Tyr219 phosphorylation leads to reductions in receptor-associated LYN, Ca²⁺ mobilization, degranulation and cytokine synthesis^{85,86}. The middle, non-canonical tyrosine (Tyr225) has some inhibitory effect on FcεRI-induced signalling through the activation of SH2-domain-containing inositol-5-phosphatase (SHIP)⁸⁵. Xiao *et al.*⁸⁷ suggested that this might be a negative-feedback mechanism that occurs during high-intensity stimulation of FcεRI.

The FcεRI-mediated calcium signal. InsP₃ is well described as a main second messenger for Ca²⁺ mobilization in immune cells. In FcεRI signalling, InsP₃ is produced mainly through PLCγ activation following SYK and BTK activation, as well as through recruitment to LAT by SLP76. BTK membrane recruitment requires binding of its pleckstrin-homology domain to the PI3K product phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P₃). InsP₃ binds to InsP₃ receptors in the ER membrane, which are Ca²⁺ channels and release

Ca²⁺ from the ER stores to the cytoplasm. Depletion of the ER stores leads to activation of plasma membrane store-operated channels (SOCs) that allow Ca²⁺ entry. The current that carries Ca²⁺ is known as *I*_{CRAC} (calcium-release-activated calcium current)⁸⁸.

Recently, we used a genome-wide screen to identify the channel producing *I*_{CRAC}. We called it **CRACM1** (REF. 89) and others have named it ORAI1 (REF. 90). This transmembrane protein multimerizes to form a SOC and cooperates with the intracellular Ca²⁺ sensor stromal interaction molecule 1 (STIM1) to mediate the store-operated Ca²⁺ influx^{91–94}.

Sphingosine 1-phosphate (S1P) is another second messenger for mast-cell function and calcium mobilization. S1P is produced by the phosphorylation of sphingosine by sphingosine kinases. Increased S1P levels are present in the airways of asthmatic subjects following localized antigen challenge, which correlates with an increase in airway inflammation⁹⁵. Furthermore, external application of S1P to mast cells induces mediator release⁹⁶. In addition, mast cells activated by FcεRI crosslinking release S1P^{96,97}, which then binds to the extracellular sphingosine receptors S1P₁ and S1P₂ and further transactivates mast cells. This creates a positive-feedback loop, which amplifies the allergic reaction by enhancing the migration of mast cells to sites of inflammation (mainly through S1P₁) and their degranulation response (mainly through S1P₂)⁹⁷.

FcεRI crosslinking induces sphingosine-kinase activity⁹⁸. This activity is responsible for a dynamic balance between sphingosine and S1P, the 'sphingolipid rheostat', with high levels of sphingosine being associated with certain signals, such as apoptosis, and high levels of S1P being associated with opposite signals, such as cell proliferation⁹⁹. This balance has been shown in particular for FcεRI (REF. 96). Although initially it was believed that sphingosine kinases, mainly through sphingosine kinase 1 (SPHK1), regulate Ca²⁺ release from intracellular stores^{98,100,101}, Olivera *et al.*¹⁰² showed that SPHK2 is the main regulator of intracellular S1P levels in mast cells and drives FcεRI-induced PKC and NF-κB activation, degranulation, lipid-mediator production and cytokine synthesis. Although SPHK2 regulates FcεRI-induced Ca²⁺ mobilization, this effect is due to the regulation of extracellular Ca²⁺ influx and not Ca²⁺ release from ER stores¹⁰². This could occur by a reduced suppression of sphingosine on *I*_{CRAC}¹⁰³ through increased conversion to S1P. Although SPHK2 seems to be the main intracellular S1P regulator in mast cells, experiments using SPHK1-deficient mice indicate that SPHK1 regulates extracellular S1P *in vivo* (probably not produced by mast cells, as mast-cell-deficient mice have normal S1P levels¹⁰²), thereby affecting mast-cell responsiveness and anaphylaxis.

The role of upstream PTK activity in the activation of sphingosine kinases has previously been investigated with controversial results^{98,101}. More recent genetic studies indicate a cooperative role for LYN and FYN in coupling SPHK1 and SPHK2 to FcεRI. LYN has a role in the early recruitment of SPHK1 to FcεRI through direct interaction, allowing its fast subsequent activation, and FYN may be responsible for prolonged sphingosine-kinase activation^{104,105}.

RABGEF1. The mitogen-activated protein kinase (MAPK) ERK signalling cascade is activated through the primary pathway by the adaptor LAT, which recruits GRB2 and the guanine-nucleotide-exchange factor son-of-sevenless homologue (SOS), which ultimately activates RAS⁷². RAS regulates FcεRI-induced lipid-mediator production (through PLA₂) and cytokine production (through transcription factors). By screening for genes that are activated after FcεRI crosslinking, Tam *et al.*¹⁰⁶ identified RAB guanine-nucleotide-exchange factor 1 (**RABGEF1**) as a novel negative regulator of FcεRI-induced signalling. RABGEF1 binds to RAS and suppresses its activation of the downstream MAPK ERK cascade. Mast cells derived from RABGEF1-deficient mice show increased lipid-mediator and cytokine production. RABGEF1-deficient mast cells also show an unexpected increase in FcεRI-induced degranulation. RAS proteins have recently been implicated in signalling pathways for FcεRI-induced degranulation. N-RAS, activated by RAS guanyl-nucleotide-releasing protein 1 (RASGRP1), might modulate degranulation through the distal complementary pathway through class I PI3K and PKCδ (REF. 107). However, the observed effect could also be due to altered differentiation of RABGEF1-deficient mast cells or compensation by other components of the FcεRI signalling pathway. RABGEF1-deficient mice have skin inflammation and increased perinatal mortality, although part of this phenotype might be due to the absence of RABGEF1 in cells other than mast cells.

NTAL. A transmembrane adaptor with structural similarities to LAT, known as **NTAL** (non-T-cell activation linker), LAB (linker for activation of B cells) or LAT2, has recently been implicated in FcεRI signalling. FcεRI crosslinking leads to rapid LYN- and SYK-dependent phosphorylation of NTAL^{108,109}. Knockdown of the gene encoding human NTAL leads to impaired mast-cell degranulation. As NTAL resides in different microdomains to LAT and the residual Ca²⁺ mobilization and degranulation observed in LAT-deficient BMMCs is further reduced in BMMCs deficient in both LAT and NTAL^{110,111}, NTAL might be the LAT counterpart for the complementary FYN pathway. However, FcεRI-induced degranulation and cytokine synthesis are upregulated in NTAL-deficient BMMCs, which indicates that NTAL is a negative regulator of FcεRI signalling. It remains to be determined whether this is a true phenomenon or whether this is due to the compensation by other FcεRI signalling molecules. Another structurally related molecule, LAX (linker for activation of X cells, where X denotes an as-yet-unidentified cell), has been proposed as a negative regulator of FcεRI signalling¹¹². So far it is unclear whether upregulation of LAX could account for the observed effect in NTAL-deficient BMMCs.

BCL-10 and MALT1. Whereas the FcεRI signalling pathways for immediate responses are relatively well defined, the signalling molecules involved in the regulation of FcεRI-induced late-phase allergic responses are less clear. The FcεRI-induced cytokine production underlying these responses is mainly the result of activation

of nuclear factor of activated T cells (NFAT), activator protein 1 (AP1) and NF-κB transcription factors^{56,113}. Although AKT is one of the upstream molecules in this pathway, especially for NF-κB and its upstream regulators IKK (inhibitor of NF-κB kinase) and IκB (inhibitor of NF-κB), it remained unclear how they were connected to FcεRI. Recently, Klemm *et al.*¹¹⁴ showed that the caspase-recruitment-domain-containing protein B-cell lymphoma 10 (**BCL-10**) and the paracaspase mucosa-associated-lymphoid tissue lymphoma-translocation protein 1 (**MALT1**) are part of this missing link. BCL-10 and MALT1 can directly bind to each other, and the two proteins cooperate in the assembly of a complex that mediates signal-specific activation of IKK in T-cell receptor signalling¹¹⁵. MALT1- or BCL-10-deficient mast cells have normal proximal FcεRI signalling and MAPK cascade activation, as well as normal degranulation and lipid-mediator release. However, FcεRI-induced NF-κB activation and production of the pro-inflammatory cytokines IL-6 and TNF were deficient. Consequently, MALT1- and BCL-10-deficient mice have an impaired IgE-mediated late-phase allergic response, whereas immediate-type allergic responses are unaffected.

Interfering with FcεRI function

FcεRI signalling and effector functions are under the control of inhibitory cell-surface molecules. Emerging new therapeutic concepts take advantage of these control mechanisms.

ITIM-containing immunoreceptors. FcεRI-mediated mast-cell and basophil effector functions are regulated by inhibitory cell-surface receptors (for a review of inhibitory receptors in allergy see REFS 116,117). Their common feature is a cytoplasmic immunoreceptor tyrosine-based inhibitory motif (ITIM). Depending on their extracellular domain, they either belong to the immunoglobulin superfamily or the C-type (calcium-dependent) lectin superfamily. Through their ITIM, these receptors suppress cell activation by promoting dephosphorylation, through phosphatases of the SH2-domain-containing protein tyrosine phosphatase (SHP) or the SHIP family (for an overview of inhibitory receptors for FcεRI-mediated activation see TABLE 2). However, the ligands for some of these receptors are unknown and their function has been detected using antibodies; therefore, their physiological relevance remains to be confirmed. For an overview on the role of PI3K, of which the product is a target for the action of many inhibitory receptors, see BOX 1.

The low-affinity IgG receptor FcγRIIB binds allergen-specific IgG and, upon co-aggregation with allergen-specific IgE bound to FcεRI, it shuts off FcεRI signalling and effector functions in mast cells and basophils¹¹⁷. The capacity of IgG to counteract IgE might be the mechanism behind successful allergen desensitization, although this has yet to be established. FcγRIIB has a broader tissue distribution than FcεRI, including B cells, and can also inhibit responses induced by IgG receptors¹¹⁷. FcγRIIB-deficient mice undergo increased FcεRI-mediated anaphylaxis, and they have

Knockdown

Reduction of expression of a gene of interest, for example through the use of short oligonucleotides, such as small interfering RNA or morpholino oligonucleotides, with sequence complementarity to a gene or a messenger RNA.

Immunoreceptor tyrosine-based inhibitory motif

(ITIM). A structural motif containing tyrosine residues that is found in the cytoplasmic tails of several inhibitory receptors, such as FcγRIIB and paired immunoglobulin-like receptor B (PIRB). The prototype six-amino-acid ITIM sequence is (Ile/Val/Leu/Ser)-X-Tyr-X-X-(Leu/Val), where X denotes any amino acid. Ligand-induced clustering of these inhibitory receptors results in tyrosine phosphorylation, often by SRC-family tyrosine kinases, which provides a docking site for the recruitment of cytoplasmic phosphatases that have an SRC homology 2 (SH2) domain.

Allergen desensitization

Activation of the immune system with increasing doses of allergens, to which a specific individual is allergic. Allergen desensitization is being used for allergies to pollen, mites, cats and insect stings. The therapy usually takes 6 months to 1 year to become effective and injections are usually required for several years.

Table 2 | Receptors that inhibit FcεRI signalling

Receptor	Ligand	Structure	Main effector(s)	References
FcγRIIB	Fc region of IgG	Immunoglobulin superfamily	SHIP1 and DOK1	78,79, 116–122
PIRB	MHC class I	Immunoglobulin superfamily	ND*	117
GP49B1	α _v β ₃ -integrin	Immunoglobulin superfamily	SHIP1	117, 151–153
MAFA (KLRG1)	ND	C-type lectin	SHIP1 and DOK1	154–158
LIR1 (CD85; ILT2)*	MHC class I	Immunoglobulin superfamily	SHIP1	159
SIRPα	CD47	Immunoglobulin superfamily	SHIP1 (and SHP2?)	160
CD300A (IRp60; LMIR1)	ND	Immunoglobulin superfamily	SHP1? SHP2?*	161
MAIR1	ND	Immunoglobulin superfamily	SHIP1? SHP1?	162
IREM1 (CD300F)†	ND	Immunoglobulin superfamily	SHP1? PI3K?	163,164

*Not directly shown for FcεRI (high-affinity Fc receptor for IgE) signalling. †FcεRI inhibitory function shown upon transfection of mast cells. Alternative receptor nomenclature is given in brackets. DOK1, docking protein 1; FcγRIIB, low-affinity Fc receptor for IgG; GP49B1, glycoprotein 49B1; IREM1, immune receptor expressed by myeloid cells 1; KLRG1, killer-cell lectin-like receptor subfamily G, member 1; IRp60, inhibitory receptor protein 60; LIR1, leukocyte immunoglobulin-like receptor 1; LMIR1, leukocyte mono-immunoglobulin-like receptor 1; MAIR1, myeloid-associated immunoglobulin-like receptor 1; MAFA, mast-cell-function-associated antigen; ND, not determined; PI3K, phosphoinositide 3-kinase; PIRB, paired immunoglobulin-like receptor B; SHP, SRC-homology-2-domain-containing protein tyrosine phosphatase; SHIP, SRC-homology-2-domain-containing inositol-5-phosphatase; SIRPα, signal regulatory protein-α.

higher immunoglobulin levels (including IgG1, which can cause anaphylactic responses in mice) and reduced nasal allergen tolerance, indicating that FcγRIIB also has a suppressive role in the sensitization phase, by inhibiting B-cell receptor activation and by downregulating APC function^{117,118}.

FcγRIIB recruits SHIP through its ITIM to the plasma membrane, where SHIP degrades PtdIns(3,4,5)P₃ to PtdIns(4,5)P₂, ultimately leading to reduced BTK activation and PLCγ-mediated Ca²⁺ mobilization through the aforementioned mechanisms^{78,79,119,120}. More recently, it has been postulated that SHIP exerts its inhibitory function through the adaptor docking protein 1 (DOK1), which then binds a RAS GTPase-activating protein that suppresses Ca²⁺ mobilization and ERK activation through an unknown mechanism^{121,122}. However, FcγRIIB can still exert its inhibitory function on FcεRI in DOK1-deficient mast cells, which might be due to compensation by a redundant molecule. Other inhibitory receptors for FcεRI are summarized in TABLE 2.

Non-ITIM-containing cell-surface molecules. Cell-surface molecules that lack ITIMs can also suppress FcεRI signalling. Antibodies specific for CD200R — a receptor for CD200 (also known as OX2) and an immunoglobulin superfamily member — suppress FcεRI-induced mast-cell degranulation and cutaneous anaphylaxis, although the mechanism remains unknown¹²³. The IgA receptor FcαRI, which associates with the γ-chain of FcεRI and therefore is an ITAM-containing receptor, can inhibit FcεRI-induced mast-cell degranulation¹²⁴. FcαRI-specific antibody Fab fragments downregulate the severity of airway inflammation and bronchial hyper-reactivity in a mouse model of asthma¹²⁴. The inhibitory mechanism seems to involve ITAM-mediated recruitment of SHP1, although a sequestration of signalling molecules from FcεRI towards FcαRI upon FcαRI activation cannot be excluded. Nevertheless, this could explain the anti-inflammatory effects of IgA and the increased susceptibility of individuals with IgA deficiency to autoimmune and allergic diseases.

Antibodies specific for the tetraspanin molecules CD63 and CD81 suppress FcεRI-induced mast-cell degranulation^{125–127}. Tetraspanins do not have known extracellular ligands or intrinsic signalling capacity, but form membrane-localized networks with each other, other tetraspanins, or other plasma-membrane proteins such as β-integrins^{128,129}. We found recently that CD63-specific antibody potently suppresses *in vivo* anaphylaxis and inhibits the complementary GAB2–PI3K pathway in mast cells while leaving FcεRI-proximal events and Ca²⁺ mobilization intact¹³⁰. β-Integrin-mediated adhesion of mast cells is known to enhance their secretory function¹³¹. CD63-specific antibody could suppress mast-cell adhesion to some β-integrin substrates and was only effective in preventing anaphylaxis in adherent mast cells. An inhibition of positive signals derived from integrins might explain the suppressive effect of the CD63-specific antibody, although the inhibition of adhesion by CD63-specific antibody could also be a secondary effect of targeting the GAB2–PI3K signalling pathway, which is used by integrins and FcεRI (REF. 130). Whether diminished mast-cell adhesion and/or migration is a component of the suppressive effect of CD63 on passive cutaneous anaphylaxis remains to be determined.

Therapeutic approaches. The inhibition of FcεRI function has led to various therapeutic approaches for allergies (for an overview see REF. 52).

Monoclonal antibodies specific for IgE are an effective adjunct therapy for asthma^{132–134}. Furthermore, there are encouraging results from clinical studies using IgE-specific antibody as a treatment for allergic rhinitis (REF. 135, among others) and peanut allergy¹³⁶. IgE-specific antibody leads to a reduction of free IgE molecules in the sera of allergic individuals, and of FcεRI levels on basophils, mast cells and DCs^{137,138}. This reduces the overall probability of IgE binding to FcεRI and of IgE–allergen contact. This therapy targets IgE molecules of any specificity, which allows its broad use but limits its efficacy. A different

Tetraspanins

A family of transmembrane proteins that have four transmembrane domains and two extracellular domains of different sizes, which are defined by several conserved amino acids in the transmembrane domains. Their function is not known clearly, but they seem to interact with many other transmembrane proteins and to form large multimeric protein networks.

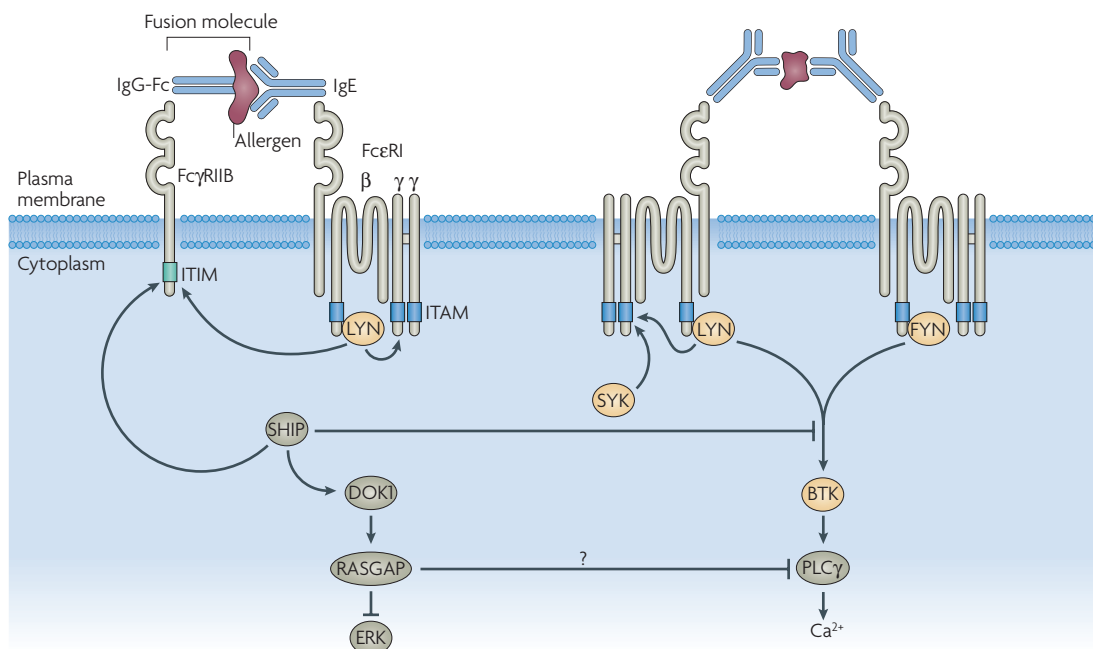


Figure 4 | Principle of antigen-specific FcεRI inhibition by a chimeric molecule consisting of an important cat allergen and IgG-Fc. Membrane-bound IgG can form immune complexes with allergens. IgG is tethered to the membrane by binding the low-affinity Fc receptor for IgG (FcγRIIB). When an allergen simultaneously binds IgE and IgG, the activating high-affinity Fc receptor for IgE (FcεRI) is brought together with the inhibitory FcγRIIB, thereby silencing the FcεRI-mediated activation pathway through the phosphatase SRC-homology-2-domain-containing inositol-5-phosphatase (SHIP1) and docking protein 1 (DOK1). Zhu and colleagues¹⁴⁴ report a strategy that takes advantage of the natural capacity of FcγRIIB to inhibit the allergenic activity of FcεRI. They designed a chimeric molecule — a fusion of a cat allergen (Fel d1) and the Fc fragment of human IgG (IgG-Fc) — that abolished allergic reactions to Fel d1 *in vitro* and in a mouse model. Similar fusion molecules could be designed to counter other types of allergy. LYN, FYN and SYK are protein tyrosine kinases. ERK, extracellular-signal-regulated kinase; BTK, Bruton's tyrosine kinase; ITAM, immunoreceptor tyrosine-based activation motif; ITIM, immunoreceptor tyrosine-based inhibitory motif; PLCγ, phospholipase Cγ; RASGAP, RAS GTPase-activating protein. Modified with permission from REF. 143 © (2005) Massachusetts Medical Society.

strategy using a chimeric fusion protein consisting of one IgE Cε3 domain and the pro-apoptotic molecule BCL-2 antagonist/killer (BAK) selectively targets and destroys FcεRI-expressing cells, such as basophils and mast cells, by the induction of apoptosis¹³⁹.

Putatively, allergen desensitization could be based on the production of allergen-specific IgG, which intercepts allergens and engages FcγRIIB, thereby turning off specific-IgG-expressing B cells and IgE-mediated responses by mast cells and basophils¹⁴⁰. However, allergen desensitization works in only a fraction of patients and can sometimes lead to anaphylactic responses. Novel approaches use the inhibitory function of FcγRIIB differently. Tam *et al.*¹⁴¹ used a bispecific antibody consisting of a human-specific IgE Fab' fragment crosslinked to a human-specific FcγRII Fab' fragment to suppress IgE–FcεRI-induced basophil and mast-cell degranulation. Zhu *et al.*¹⁴² reported that a chimeric molecule consisting of the Fc portion of human IgG1 fused to the Fc portion of human IgE inhibits FcεRI-induced degranulation. Recently, they refined the chimeric molecule such that it contains human IgG1 Fc and the cat allergen Fel d1 (REFS 143,144) (FIG. 4). This molecule suppresses mast-cell and basophil degranulation by allergen-specific

IgE and cat allergen, as well as allergen-mediated anaphylaxis and airway inflammation and bronchial hyper-reactivity in human FCER1A-transgenic mice. It cannot be excluded that the observed effect is partially due to competition of the chimeric molecule with Fel d1 allergen for binding to IgE and not to FcγRIIB-mediated suppression. For example, the authors report suppressed SYK activation, an upstream event that should be unaffected by FcγRIIB-mediated inhibition. However, the authors used cord-blood-derived mast cells, for which the FcγRIIB inhibitory mechanism through SHIP and DOK1 has not been investigated. This novel approach might not only work for cat allergy, but also provides proof of principle for other allergies such as peanut allergies, which can be life-threatening and for which no effective allergen desensitization protocol exists¹³⁶. It might suppress not only the effector cells of allergy, but also the production of IgE by co-ligating FcγRIIB on specific-IgE-expressing B cells or plasma cells, thereby leading to growth arrest and deletion of specific clones. Although the chimeric molecule by itself cannot activate mast-cell degranulation, it might be harmful in a subgroup of patients in which activating FcγRIIA prevails over FcγRIIB, as in the case of polymorphisms of the FCGR1B gene¹⁴⁵.

Conclusions

In this Review, we discuss recent developments in the regulation of FcεRI expression, novel biological functions of FcεRI that are antigen (allergen) independent, and the increasingly complex topic of FcεRI signalling and negative regulation. Despite these advances, questions remain. For example, the recent finding of an involvement of mast cells in autoimmune diseases¹⁴⁶ raises the question of a possible role for FcεRI in some of these diseases. In addition, it would be interesting to

know more about the mechanisms by which FcεRI on mast cells might contribute to contact hypersensitivity and colitis. The still enigmatic role of FcεRI on APCs in humans is another area in which additional data are required. Finally, the increase in the number of known FcεRI signalling molecules increases the number of possible drug targets, but as the signalling molecules are often redundant, careful selection of drug targets that are essential for the function of FcεRI is required.

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Competing interests statement

The authors declare no competing financial interests.

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